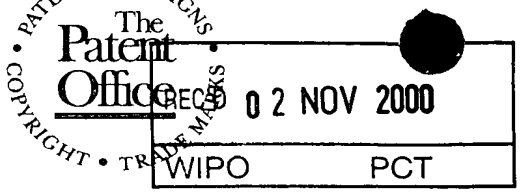
PCT/EP00/08784

#2



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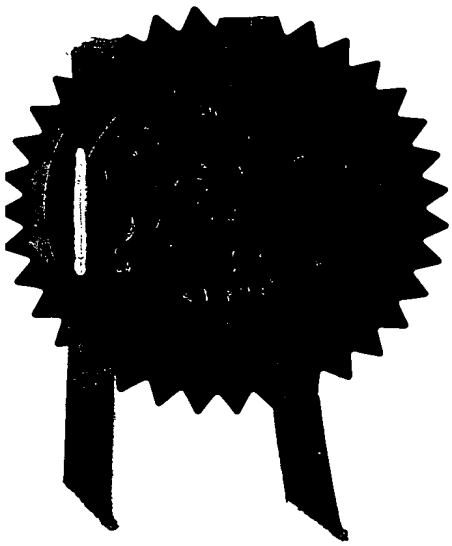

Signed R. Mahoney

Dated 22nd August 2000

-7 SEP 1999

Fo ficial use

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-7 SEP 1999

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P01/7700 0.00 - 9921146.8

Your Reference: KLP/RH/ B45198

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The Patent Office

Request for grant of a Patent

Form 1/77 **Patents Act 1977**

① Title of invention

1 Please give the title of the invention: Novel Composition

② Applicant's details

❑ **First or only applicant**

2a If you are applying as a corporate body please give:
Corporate Name: SmithKline Beecham Biologicals s.a.

Country *(and State of incorporation, if appropriate)*: Belgium

2b If you are applying as an individual or one of a partnership please give in full:

Surname

Forenames

2c **In all cases,** please given the following details:

Address: rue de l'Institut 89, B-1330 Rixensart
Belgium

postcode *(if applicable)* 5781117001

Country: Belgium

ADP number *(if known)*: ~~5800974002~~

Reference number

4. Agent's or applicant's reference number *(if applicable)* KLP/RH B45198

⑤ Claiming an earlier application date

5. Are you claiming that this application be treated as having been filed on the date of filing of an earlier application?

Please mark correct box

Yes ☐ No ☒ *go to 6*

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☐ filing date *(day month year)*

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15(4) (Divisional) ☐ 8(3) ☐ 12(6) ☐ 37(4) ☐

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If you are declaring priority from a PCT Application please enter 'PCT' as the country and enter the country code (for example, GB) as part of the application number

Please give the date in all number format, for example, 31/05/90 for 31 May 1990

6. If you are declaring priority from previous application(s), please give:

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Novel Composition

This invention relates to novel vaccine formulations, methods for preparing them and their use in therapy. In particular the present invention relates to combination vaccines for administration to adolescents.

Papillomaviruses are small DNA tumour viruses, which are highly species specific. So far, over 70 individual human papillomavirus (HPV) genotypes have been described. HPVs are generally specific either for the skin (e.g. HPV-1 and -2) or mucosal surfaces (e.g. HPV-6 and -11) and usually cause benign tumours (warts) that persist for several months or years. Such benign tumours may be distressing for the individuals concerned but tend not to be life threatening, with a few exceptions.

Some HPVs are also associated with cancers. The strongest positive association between an HPV and human cancer is that which exists between HPV-16 and HPV-18 and cervical carcinoma. Cervical cancer is the most common malignancy in developing countries, with about 500,000 new cases occurring in the world each year. It is now technically feasible to actively combat primary HPV-16 infections, and even established HPV-16-containing cancers, using vaccines. For a review on the prospects for prophylactic and therapeutic vaccination against HPV-16 see Cason J., Clin. Immunother. 1994; 1(4) 293-306 and Hagenesee M.E., Infections in Medicine 1997 14(7) 555-556,559-564.

Other HPVs of particular interest are serotypes 31,33 and 45.

Today, the different types of HPVs have been isolated and characterised with the help of cloning systems in bacteria and more recently by PCR amplification. The molecular organisation of the HPV genomes has been defined on a comparative basis with that of the well-characterised bovine papillomavirus type 1 (BPV1).

carcinoma in situ (CIS) which are themselves regarded as precursor lesions of invasive cervix carcinoma.

WO 96/19496 discloses variants of human papilloma virus E6 and E7 proteins, particularly fusion proteins of E6/E7 with a deletion in both the E6 and E7 proteins. These deletion fusion proteins are said to be immunogenic.

HPV L1 based vaccines are disclosed in WO94/00152, WO94/20137, WO93/02184 and WO94/05792. Such a vaccine can comprise the L1 antigen as a monomer, a capsomer or a virus like particle. Such particles may additionally comprise L2 proteins. L2 based vaccines are described for example in WO93/00436. Other HPV vaccines are based on the Early proteins, such as E7 or fusion proteins such as L2-E7.

HSV-2 is the primary etiological agent of herpes genitalis. HSV-2 and HSV-1 (the causative agent of herpes labialis) are characterised by their ability to induce both acute diseases and to establish a latent infection, primarily in neuronal ganglia cells.

Genital herpes is estimated to occur in about 5 million people in the U.S.A. alone with 500,000 clinical cases recorded every year (primary and recurrent infection). Primary infection typically occurs after puberty and is characterised by the localised appearance of painful skin lesions, which persist for a period of between 2 to 3 weeks. Within the following six months after primary infection 50% of patients will experience a recurrence of the disease. About 25% of patients may experience between 10-15 recurrent episodes of the disease each year. In immunocompromised patients the incidence of high frequency recurrence is statistically higher than in the normal patient population.

Both HSV-1 and HSV-2 virus have a number of glycoprotein components located on the surface of the virus. These are known as gB, gC, gD and gE etc.

attenuated strain of the HM-175 Hepatitis A virus inactivated with formol (formaldehyde); see Andre et. al. (Prog. med. Virol., vol. 37, p1-24).

As used herein, the term hepatitis A viral (HAV) antigen is used to refer to either a protein derived from hepatitis A virus or an attenuated strain of HAV, optionally inactivated, e.g. with formaldehyde. If the HAV antigen is a protein derived from hepatitis A virus it may optionally be a recombinant protein.

The vaccine Twinrix (Trade Mark) is a combination of a recombinant hepatitis B anitgen with the aforementioned inactivated attenuated hepatitis A virus. The vaccine may be used to protect against hepatitis A and hepatitis B simultaneously.

European patent 0 339 667 (Chemo Sero) describes the general concept of combining a hepatitis A antigen and a hepatitis B antigen to make a combination vaccine. In that specification it is stated that the adjuvant which is used is not critical: it must only be capable of enhancing the immune activity to a desired extent and not cause any side-effects. It is stated that aluminium gel may be used, in particular aluminium hydroxide gel and aluminium phosphate gel.

The vaccine composition according to the invention may comprises, in addition the HPV and HSV antigens, an HAV antigen or a HBV antigen or more preferably a combination of both an HAV and an HBV antigen

Such a vaccine is of great benefit for administration to adolescents who may be particularly at risk of HSV, and/or HPV infection, and/or HAV infection, and/or HBV infection.

An immune response may be broadly divided into two extreme catagories, being a humoral or cell mediated immune response (traditionally characterised by antibody and cellular effector mechanisms of protection respectively). These categories of response have been termed TH1-type responses (cell-mediated response), and TH2-type immune responses (humoral response).

in vitro after restimulation with antigen, and/or (at least in mice) the measurement of the IgG1:IgG2a ratio of antigen specific antibody responses.

Thus, a TH1-type adjuvant is one which stimulates isolated T-cell populations to produce high levels of TH1-type cytokines when re-stimulated with antigen *in vitro*, and induces antigen specific immunoglobulin responses associated with TH1-type isotype.

Adjuvants which are capable of preferential stimulation of the TH1 cell response are described in International Patent Application No. WO 94/00153 and WO 95/17209.

3 De-O-acylated monophosphoryl lipid A (3D-MPL) is one such adjuvant. This is known from GB 2220211 (Ribi). Chemically it is a mixture of 3 De-O-acylated monophosphoryl lipid A with 4, 5 or 6 acylated chains and is manufactured by Ribi Immunochem, Montana. A preferred form of 3 De-O-acylated monophosphoryl lipid A is disclosed in European Patent 0 689 454 B1 (SmithKline Beecham Biologicals SA).

Preferably, the particles of 3D-MPL are small enough to be sterile filtered through a 0.22micron membrane (as described in European Patent number 0 689 454). 3D-MPL will be present in the range of 10μg - 100μg preferably 25-50μg per dose wherein the antigen will typically be present in a range 2-50μg per dose.

Another preferred adjuvant comprises QS21, an Hplc purified non-toxic fraction derived from the bark of Quillaja Saponaria Molina. Optionally this may be admixed with 3 De-O-acylated monophosphoryl lipid A (3D-MPL), optionally together with an carrier.

The method of production of QS21 is disclosed in US patent No. 5,057,540.

Non-reactogenic adjuvant formulations containing QS21 have been described previously (WO 96/33739). Such formulations comprising QS21 and cholesterol

advantageous that the vaccines of the present invention will further contain a stabiliser.

Non-toxic oil in water emulsions preferably contain a non-toxic oil, e.g. squalane or squalene, an emulsifier, e.g. Tween 80, in an aqueous carrier. The aqueous carrier may be, for example, phosphate buffered saline.

A particularly potent adjuvant formulation involving QS21, 3D-MPL and tocopherol in an oil in water emulsion is described in WO 95/17210.

The HPV antigen in the composition of the invention is preferably derived from HPV 16 and/or 18, or from HPV 6 and/or 11, or HPV 31, 33 or 45.

In one preferred embodiment the HPV antigen in the vaccine composition according to the invention comprises the major capsid protein L1 of HPV and optionally the L2 protein, particularly from HPV 16 and/or HPV 18. In this embodiment, the preferred form of the L1 protein is a truncated L1 protein. Preferably the L1, optionally in a L1-L2 fusion, is in the form of a virus-like particle (VLP). The L1 protein may be fused to another HPV protein, in particular E7 to form an L1-E7 fusion. Chimeric VLPs comprising L1-E or L1-L2-E are particularly preferred.

In another preferred embodiment, the HPV antigen in the composition of the invention is derived from an E6 or E7 protein, in particular E6 or E7 linked to an immunological fusion partner having T cell epitopes.

In a preferred form of this embodiment of the invention, the immunological fusion partner is derived from protein D of *Heamophilus influenza* B. Preferably the protein D derivative comprises approximately the first 1/3 of the protein, in particular approximately the first N-terminal 100-110 amino acids.

Preferred fusion proteins in this embodiment of the invention comprise Protein D - E6 from HPV 16, Protein D - E7 from HPV 16 Protein D - E7 from HPV 18 and

The recombinant mature HSV-2 glycoprotein D truncate is preferably used in the vaccine formulations of the present invention and is designated rgD2t.

A combination of this HSV-2 antigen in combination with the adjuvant 3D-MPL has been described in WO 92/16231

When a hepatitis B viral (HBV) antigen is included in the composition of the invention this is typically hepatitis B surface antigen.

The preparation of Hepatitis B surface antigen (HBsAg) is well documented. See for example, Harford et.al. in Develop. Biol. Standard 54, page 125 (1983), Gregg et.al. in Biotechnology, 5, page 479 (1987), EP-A- 0 226 846, EP-A-0 299 108 and references therein.

As used herein the expression 'Hepatitis B surface antigen', abbreviated herein to 'HBsAg' or 'HBS' includes any HBsAg antigen or fragment thereof displaying the antigenicity of HBV surface antigen. It will be understood that in addition to the 226 amino acid sequence of the HBsAg S antigen (see Tiollais et. al. Nature, 317, 489 (1985) and references therein) HBsAg as herein described may, if desired, contain all or part of a pre-S sequence as described in the above references and in EP-A- 0 278 940. HBsAg as herein described can also refer to variants, for example the 'escape mutant' described in WO 91/14703. In a further aspect the HBsAg may comprise a protein described as L* in European Patent Application Number 0 414 374, that is to say a protein, the amino acid sequence of which consists of parts of the amino acid sequence of the hepatitis B virus large (L) protein (ad or ay subtype), characterised in that the amino acid sequence of the protein consists of either:

(a) residues 12 - 52, followed by residues 133 - 145, followed by residues 175 - 400 of the said L protein; or

(b) residue 12, followed by residues 14 - 52, followed by residues 133 - 145, followed by residues 175 - 400 of the said L protein.

lymphomas, including African Burkitt's lymphoma (BL). EBV may also be involved in causing nasopharyngeal carcinoma (NPC). Worldwide it is estimated that 80,000 cases of nasopharyngeal carcinoma occur and it is more prevalent in ethnic Chinese populations. Infectious mononucleosis is a consequence of primary infection by EBV. It is not a life-threatening disease if additional risk factors are absent.

Four proteins of the EBV viral envelope constituting the so-called membrane antigen complex have been described. They are usually referred to as gp 220/350 or gp 250/350 or simply as gp 250 or 350 (see EP-A-151079). There is convincing evidence that gp 350 and gp 250 induce the production of neutralising antibodies and that antibodies against gp 350 and gp 250 have neutralising capacity. These proteins are thus candidates for a possible EBV vaccine. For further information about the application of gp 250/350 for prophylaxis and treatment of EBV-related diseases see EP 0 173 254.

The major EBV surface glycoprotein gp350/220 infects human target cells through interaction with the cellular membrane protein, CD21. Gp350/220 is the primary target for EBV-neutralising antibodies in humans and some forms of gp350/220 have been shown to protect against EBV-related disease. Preferably a vaccine composition according to the invention comprises gp 350 of EBV although other protective antigens may be used.

In a preferred aspect the vaccine composition of the invention additionally comprises a Varicella Zoster viral antigen (VZV antigen). Suitable antigens of VZV for inclusion in the vaccine formulation include gpI-V described by Longnecker et al., Proc Natl Acad Sci USA 84, 4303-4307 (1987).

In a preferred embodiment gpI (see Ellis et al., US patent 4,769,239) is used. See also European Patent No. 0 405 867 B1.

The formulations of the present invention are very effective in inducing protective immunity, even with very low doses of antigen (e.g. as low as 5μg rgD2t).

They provide excellent protection against primary infection and stimulate, advantageously both specific humoral (neutralising antibodies) and also effector cell mediated (DTH) immune responses.

The present invention in a further aspect provides a vaccine formulation as herein described for use in medical therapy, particularly for use in the treatment or prophylaxis of human papillomavirus infections and herpes simplex virus infections.

The vaccine of the present invention will contain an immunoprotective quantity of the antigens and may be prepared by conventional techniques.

Vaccine preparation is generally described in Pharmaceutical Biotechnology, Vol.61 Vaccine Design - the subunit and adjuvant approach, edited by Powell and Newman, Plenurn Press, 1995. New Trends and Developments in Vaccines, edited by Voller et al., University Park Press, Baltimore, Maryland, U.S.A. 1978. Encapsulation within liposomes is described, for example, by Fullerton, U.S. Patent 4,235,877. Conjugation of proteins to macromolecules is disclosed, for example, by Likhite, U.S. Patent 4,372,945 and by Armor et al., U.S. Patent 4,474,757.

The amount of protein in each vaccine dose is selected as an amount which induces an immunoprotective response without significant, adverse side effects in typical vaccinees. Such amount will vary depending upon which specific immunogen is employed. Generally, it is expected that each dose will comprise 1-1000μg of protein, preferably 2-100μg, most preferably 4-40μg. An optimal amount for a particular vaccine can be ascertained by standard studies involving observation of antibody titres and other responses in subjects. Following an initial vaccination, subjects may receive a boost in about 4 weeks.

CLAIMS

1. A vaccine composition comprising:

(a) a herpes simplex virus (HSV) antigen; and

(b) a human papillomavirus (HPV) antigen

in conjunction with an adjuvant which is a preferential stimulator of TH1 cell response.

2. A vaccine composition according to claim 1 which additionally comprises a carrier.

3. A vaccine composition according to claim 1 or claim 2 in which the preferential stimulator of TH1-cell response is selected from the group of adjuvants comprising: 3D-MPL, 3D-MPL wherein the size of the particles of 3D-MPL is preferably about or less than 100nm, QS21, a mixture of QS21 and cholesterol, and a CpG oligonucleotide.

4. A vaccine composition according to claim 3 in which the preferential stimulator of TH1-cell response is 3D-MPL.

5. A vaccine compostion according to any one of claims 1 to 4 in which the HSV antigen is HSV-2 gD or a truncate thereof.

6. A vaccine composition according to any one of claims 1 to 5 which comprises at least one HPV antigen selected from the group consisting of L1, L2, E6 and E7, optionally in the form of a fusion protein and/or a truncate.

7. A vaccine composition according to any one of claims 1 to 6 in which an EBV antigen is additionally present.

8. A vaccine composition as defined in claim 7 in which the EBV antigen is gp 350.

19. A vaccine composition according to claim 18 in which the *Toxoplasma gondii* antigen is SAG1 or TG34.

20. A vaccine composition according to any one of claims 1 to 4 comprising HSV-2 gDt antigen and an HPV antigen chosen from the group consisting of L1, L2, E6, E7, protein D-E6, protein D-E7 or L2-E7 of HPV and optionally in addition one or more of HBsAg S antigen; EBVgp 350; VZVgpI; HAV HM-175 inactivated strain; gB685** or pp65 of HCMV and SAG1 or TG34 antigens of *Toxoplasma gondii*.